ABSTRACT

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The production of secondary metabolites in the *Trifolium pratense* L. suspension culture

Summary

Elicitation is a method making use of protective mechanisms of plants to increase the production of secondary metabolites in plants and cultures *in vitro*. Jasmonic acid we can include into biotic elicitors, because it acts in plants as an inducer of the expression of protective proteins. In the experiment examined the impact of four concentrations of jasmonic acid and the impact of jasmonic acid in combination with calcium ions and verapamil on the production of flavonoids and isoflavonoids by the *Trifolium pratense* L. suspension culture (variety Sprint).

The culture was cultivated in Gamborg medium to which 2 $mg.l^{-1}$ of 2,4-dichlorophenoxyacetic acid and 2 $mg.l^{-1}$ of 6-benzylaminopurine were added, at the temperature of 25°C, 16-hr light/8-hr dark period. The elicited and the inspection samples underwent the photometric determination of flavonoids in accordance with the Czech Pharmacopoeia 2009 and the determination of isoflavonoids via the HPLC method. The results show that the optimal effect of jasmonic acid on the production of flavonoids and isoflavonoids was manifested after a 24-hour application of the 50 μmol concentration. The stimulation of elicitation with extracellular calcium ions mostly took effect in the frailest concentration of jasmonic acid (5 μmol) after the addition of the 1 and 10 mmol concentration $CaCl_2$. An application of verapamil decreased production of flavonoids blockage calcium channels.